

-continued

```

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Signal Sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(66)

<400> SEQUENCE: 1

atg gac atg agg gtg ccc gct cag ctc ctg ggg ctc ctg ctg ctg tgg      48
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1          5          10          15

ctg aga ggt gcg cgc tgt      66
Leu Arg Gly Ala Arg Cys
20

<210> SEQ ID NO 2
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 2

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1          5          10          15

Leu Arg Gly Ala Arg Cys
20

```

1-10. (canceled)

11. An in vitro method of producing a recombinant AAV virion in a mammalian host cell, wherein the mammalian host cell is a HEK 293T cell, and wherein the cell is suspended in a transfection media, comprising contacting the cell with polyethylenimine ("PEI") and:

- (i) an accessory construct, wherein said accessory construct is a plasmid (pHelper) consisting essentially of adenoviral E2, E4Orf6, and VAI RNA genes operably linked to an origin of replication element and one or more other regulatory sequences;
- (ii) an AAV helper construct, wherein said AAV helper construct is a plasmid (pTrans) consisting essentially of AAV2 rep and AAV1, AAV2, AAV5, AAV8, or AAV9 cap coding regions operably linked to one or more regulatory sequences; and
- (iii) an AAV vector construct, wherein said AAV vector construct is a plasmid (pCis) consisting essentially of AAV2 inverted terminal repeats flanking a heterologous gene of interest operably linked to one or more regulatory sequences,

wherein the ratio of pHelper:pTrans:pCis is 1:5:(0.009-0.36) (weight:weight:weight), the PEI is Transfection Grade Linear Polyethylenimine Hydrochloride (MW 40,000), and the ratio of PEI:DNA is about 3:1.

12. The method of claim **11**, wherein the HEK 293T cell is suspended at a cell density of $2.1\text{--}3.0 \times 10^6$ cells/mL.

13. The method of claim **12**, wherein the HEK 293T cell is suspended at a cell density of $2.2\text{--}2.7 \times 10^6$ cells/mL.

14. The method of claim **13**, wherein the HEK 293T cell is suspended at a cell density of about 2.5×10^6 cells/mL.

15. The method of claim **11**, wherein the ratio of pHelper:pTrans:pCis is 1:5:(0.30-0.36) (weight:weight:weight).

16. The method of claim **15**, wherein the ratio of pHelper:pTrans:pCis is 1:5:0.31 (weight:weight:weight).

17. The method of claim **11**, wherein the total amount of plasmid does not exceed 1.5 mg/L of transfection media.

18. An in vitro method of producing a recombinant AAV virion in a mammalian host cell, wherein the mammalian host cell is a HEK 293T cell, and wherein the cell adheres to a solid substrate in a transfection media, comprising contacting the cell with calcium phosphate and:

- (i) an accessory construct, wherein said accessory construct is a plasmid (pHelper) consisting essentially of adenoviral E2, E4Orf6, and VAI RNA genes operably linked to an origin of replication element and one or more other regulatory sequences;
- (ii) an AAV helper construct, wherein said AAV helper construct is a plasmid (pTrans) consisting essentially of AAV2 rep and AAV1, AAV2, AAV5, AAV8, or AAV9 cap coding regions operably linked to one or more regulatory sequences; and
- (iii) an AAV vector construct, wherein said AAV vector construct is a plasmid (pCis) consisting essentially of AAV2 inverted terminal repeats flanking a heterologous gene of interest operably linked to one or more regulatory sequences,

wherein the ratio of pHelper:pTrans:pCis is 1:5:(0.009-0.36) (weight:weight:weight).

19. The method of claim **18**, wherein the HEK 293T cell is seeded at a cell density of $2.1\text{--}3.0 \times 10^6$ cells/mL.

20. The method of claim **12**, wherein the HEK 293T cell is seeded at a cell density of $2.2\text{--}2.7 \times 10^6$ cells/mL.

21. The method of claim **13**, wherein the HEK 293T cell is seeded at a cell density of about 2.5×10^6 cells/mL.